

# Biodegradable-co-bioresponsive Hydrogels for Controlled Release of Growth Factor

X. Huang\*, B. R. Nayak, Department of Surgery; T.L. Lowe, Department of Surgery, Bioengineering and Materials Science and Engineering, Pennsylvania State University, Hershey, PA 17033

## Abstract

A series of multi-functional hydrogel systems have been designed and synthesized for potential brain implantation to realize controlled and sustained release of Nerve Growth Factor (NGF) for the treatment of Alzheimer's disease (AD). The systems are three-dimensional crosslinked copolymers composed of a thermo-responsive unit, a hydrolytically degradable and hydrophobic unit, and an enzymatically degradable and hydrophilic unit. They combine the merits of thermo-responsive and biodegradable polymeric drug delivery systems, and allow a low-temperature aqueous NGF loading to prevent protein denaturation. The hydrogels show a lower critical solution temperature (LCST) at approximately 32 °C, and demonstrate different swelling and release profiles at temperatures above (37 °C) and below (25 °C) the LCST.

## Introduction

Degeneration of specific cell populations is a frequent finding in disorders of the central nervous system (CNS) such as Alzheimer's disease. Administration of nerve growth factor has been found to promote the survival and neurite outgrowth of degenerating cholinergic neurons and provide treatment to AD<sup>1</sup>. However, the systemic administration of the NGF remains a dilemma because growth factors typically have half-lives only on the order of minutes. In addition, they do not readily cross the blood brain barrier (BBB) and are easily metabolized at multiple tissue sites<sup>1, 2</sup>. Under these circumstances, brain implantation of polymeric controlled release devices emerges as a promising method to realize the local and sustained delivery of NGF<sup>2</sup>.

Biodegradable polymers play an important role in the sustained delivery of the NGF<sup>2</sup>. These polymers degrade by hydrolytic or enzymatic cleavage of the backbone after certain period of time; hence can avoid the surgical removal after the drug depletion. However, many currently investigated biodegradable polymers have disadvantages in the fabrication processes involving organic solvents, inconsistent drug release kinetics, and lack of response to physiological changes in living bodies<sup>3,4</sup>. Bioresponsive polymers are another class of polymers widely studied, especially as devices for the delivery of physiological unstable agents, such as protein drugs including growth factors<sup>5</sup>. This class of materials is responsive to physical, chemical, or biological stimuli. They can protect the drug from a hostile environment and allow aqueous loading of drugs with high loading efficiency<sup>6</sup>. However, the responsive polymers have problems in non-biodegradability. A promising strategy for designing novel drug delivery systems is based on combining the merits of the above two polymeric systems, which are both biodegradable and responsive to physiological stimuli<sup>7,8</sup>.

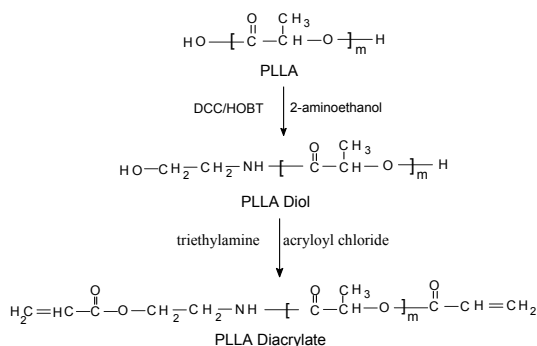
Hydrogels are crosslinked three-dimensional polymer networks highly swollen in aqueous conditions. Due to their high water content, hydrogels resemble the body tissues more than other materials. As drug delivery devices, hydrogels show advantages in controlling release profiles by changing their chemical and physical properties<sup>9</sup>. In our studies, we have designed and developed a series of multi-functional hydrogels systems with thermo-responsive and biodegradable properties. The novel hydrogels are crosslinked copolymers composed of poly(L-lactic acid) (PLLA), dextran and N-isopropylacrylamide (NIPAAm) units. Poly(lactic acid), a biodegradable hydrophobic polymer, has been used widely for structural support and as a hydrophobic drug carrier because of its combination of biodegradability, biocompatibility, and good mechanical strength<sup>10</sup>. Dextran, a hydrophilic natural polysaccharide, attracted much attention for use in a controlled drug delivery system when it is incorporated into poly(lactic acid) because of its excellent hydrophilic nature and biocompatibility<sup>11</sup>. Poly(N-isopropylacrylamide) (PNIPAAm) has been extensively investigated for controlled release of drugs due to its unique thermosensitive properties in water<sup>12</sup>. This "intelligent" polymer exhibits a dramatic solubility transition at the lower critical solution temperature (LCST) in an aqueous solution in the vicinity of 32 °C. It expands and swells when cooled below the LCST, and shrinks and collapses when heated above the LCST. The LCST as well as the environmental responsive properties of the polymers may be manipulated by changing the polymer compositions<sup>9, 13</sup>. Due to the rational design and the multi-functional properties, the designed hydrogel systems in this work show great potential for controlled and sustained NGF release for the treatment of Alzheimer's disease.

## Experimental Section

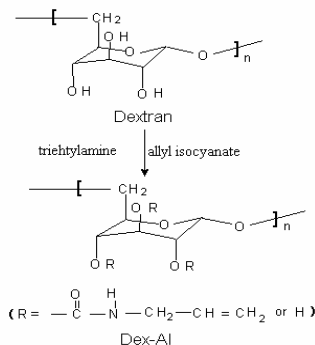
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## Synthesis of hydrogels

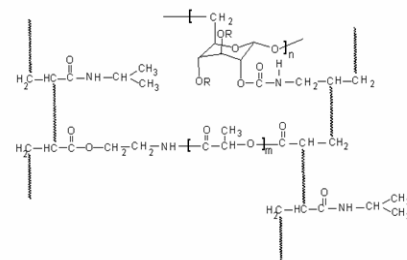
To prepare the hydrogels, PLLA and dextran had to be modified chemically so that they had the required unsaturated functional groups to undergo crosslinking<sup>14, 15</sup>. Therefore, the synthesis of the hydrogels includes three steps: (1) modify PLLA (molar mass 2000 g·mol<sup>-1</sup>) as a PLLA diacrylate macromer, (2) modify dextran (molar mass 1500 g·mol<sup>-1</sup>) as a dextran allyl isocyanate (DAI) macromer, and (3) chemically crosslink NIPAAm, PLLA diacrylate macromer, and DAI macromer to form copolymeric hydrogels.



Scheme 1 Synthesis of PLLA dial and PLLA diacrylate macromer from PLLA



Scheme 2 Synthesis of DAI macromer from dextran



Scheme 3 Structure of NIPAAm-co-PLLA-co-dextran hydrogels

Four types of hydrogel were synthesized in DMF under stirrer at 70 °C by solution polymerization for four hours, using azobisisobutyronitrile as an initiator, N,N'-methylene-bis-acrylamide as a crosslinker, and N,N,N',N'-tetramethylethylenediamine as an accelerator. Their compositions are summarized in Table 1. Two sizes of samples were cut. The larger samples, around 8 mm in diameter and 3 mm in thickness, were used in swelling studies. The smaller samples, approximately 3 mm in diameter and 1 mm in thickness, were used in NGF release studies. The hydrogels were further purified several times with deionized water and ethanol mixture (50:50 v/v) for one day and dried at room temperature for one week.

Table 1 Compositions of hydrogels

Samples	Monomer Feeding Molar Ratios		
	NIPAAm	L-lactic acid unit in PLA	Anhydroglucose unit in dextran
PNIPAAm	100	0	0
90:10	90	10	0
80:15:5	80	15	5
93:5:2	93	5	2

The chemical structures of the hydrogels were verified by FTIR. The thermoresponsive properties of the hydrogels with 8 mm diameter were studied by measuring their swelling ratios as a function of temperature from 10 to 60 °C after incubation for 1 day at each temperature interval in PBS solvent (pH=7.4). The swelling kinetics of the hydrogels in PBS solvent was investigated at 25 and 37 °C, respectively. The swelling ratio was calculated using formula: Swelling ratio = (W<sub>s</sub>-W<sub>d</sub>)/W<sub>d</sub>, where W<sub>s</sub> is the weight of the swollen hydrogel at a particular temperature or time point, and W<sub>d</sub> is the weight of the dry sample. For NGF loading, the dried hydrogels with 3 mm diameter were swollen in PBS solvent at 10 °C for one day, then transferred into PBS solution (pH=7.4) of NGF (concentration of 25 µg·ml<sup>-1</sup>, containing 80:1 (w/w) BSA) to load at 10 °C for one day, and then dried in the air. The NGF-loaded hydrogels were placed into PBS solvent containing 1% (w/v) BSA to perform release studies at 25 and 37 °C. The amount of NGF released as a function of time was determined by ELISA assay. The morphology of hydrogels was investigated by Scanning Electron Microscopy (SEM) to study the degradation process. Samples immersed in PBS solvent at 37 °C for certain period of time were dipped into liquid nitrogen immediately and freeze-dried overnight. Both the freeze-dried samples and the dry hydrogel samples at initial state after synthesis were coated with gold and observed under SEM.

## Results and Discussion

### Thermo-responsive Properties of the Hydrogels

Figure 1 shows the thermoresponsive properties of the hydrogels by their swelling ratios as a function of temperature from 10 to 60 °C in PBS solvent (pH=7.4). The swelling ratios of all the hydrogels decreased with increasing temperature and showed

the lower critical solution temperature (LCST) around 32 °C. The chemical compositions of the copolymers had no significant effect on the LCST. Below the LCST, when hydrophobic PLLA segments (copolymeric hydrogel 90:10) were added into PNIPAAm, the swelling ratio significantly decreased about one fold. When hydrophilic dextran segments (copolymeric hydrogels 80:15:5 and 93:5:2) were further added, the swelling ratios of both hydrogels 80:15:5, and 93:5:2 were higher than that of the hydrogel 90:10 but lower than that of the homohydrogel PNIPAAm. The higher was the ratio of dextran segments to PLLA segments, the higher was the swelling ratio (the swelling ratio of the hydrogel 93:5:2 was higher than that of the hydrogel 80:15:5). In contrast and interestingly, above the LCST, the swelling ratios of both copolymeric hydrogel 80:15:5 and 93:5:2 were higher than that of the homohydrogel PNIPAAm and decreased with the increase of total amount of dextran segments. The reason may be that the hydrogels collapsed above the LCST, and the hydrophilic dextran segments of the hydrogels were exposed to the aqueous environment; therefore, hydrogels with higher amount of dextran segments swelled more.

### Swelling Kinetics of the Hydrogels

Figure 2 shows the swelling kinetics of the four hydrogels at the temperatures below the LCST at 25 °C. All the hydrogels swelled rapidly within first 12 h and reached swelling equilibrium within two days. The swelling kinetics of the hydrogels was strongly depended on the molar ratios of the hydrophobic L-lactic acid unit in PLLA and anhydroglucose unit in dextran, higher molar ratio brought slower hydrogel swelling, for example, the swelling speed decreased in PNIPAAm > 93:5:2 > 80:15:5 > 90:10.

In contrast, the swelling behaviors of the same hydrogels at the temperature above the LCST at 37 °C were significantly different from those at 25 °C, as shown in Figure 3. At 37 °C, the PNIPAAm and 90:10 gels only swelled slightly, and the equilibrium swelling ratios were reached within hours. With a small portion of hydrophilic dextran component, the 93:5:2 gel kept swelling at a low rate after an initial fast swelling similar to the PNIPAAm and 90:10 gels. However, the 80:15:5 gel demonstrated dramatically different swelling behavior from the other three gels with a short rapid swelling at the beginning followed by a near-linear swelling kinetics.

### Biodegradable Properties of the Hydrogels

The design of the novel hydrogel systems includes a PLLA backbone, which is hydrolytically degradable by the cleavage of ester bonds. The SEM studies (pictures not shown) suggested that dry hydrogels in the initial state had smooth surfaces. After immersion in PBS (pH 7.4) solvent for approximately four months, the 90:10, 80:15:5, and 93:5:2 gels presented pores, cracks, or loosened 3D structures of different degrees, which were attributed to the degradation of the PLLA backbones. On the other hand, the PNIPAAm gels kept a compact network structure with much smaller pores in irregular shapes, which were due to the polymer swelling and chain relaxation in the buffer solvent.

### In vitro NGF Release Study

In vitro NGF release experiments were conducted at temperatures both above (37 °C) and below (25 °C) the LCST. In both situations the release was characterized by an initial burst effect followed by a low rate sustained release; representative release experimental data are shown in Table 2. The 37 °C results indicate that the release kinetics under this condition was primarily controlled by the hydrogel swelling process, as the release profiles were in the similar trends as the swelling profiles. In contrast, release kinetics at below the LCST 25 °C appeared to be influenced by multiple factors. Although PNIPAAm gels had the highest swelling ratio under this condition, their NGF release rate was only slightly higher than the 90:10 samples among the four types of hydrogels. The two dextran-containing gels, 80:15:5 and 93:5:2 gels, released NGF at higher rates comparing to the other two dextran-free gels. These experimental results suggest that below the LCST at 25 °C,

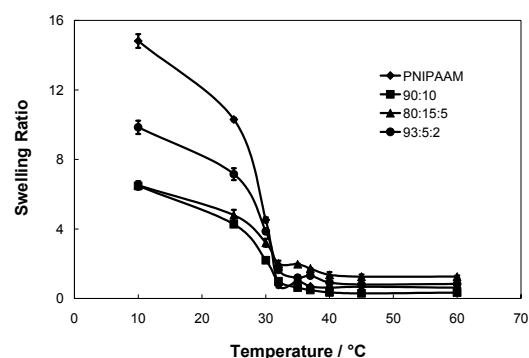


Figure 1 Swelling ratios of the hydrogels as a function of temperature in PBS solution (pH 7.4).

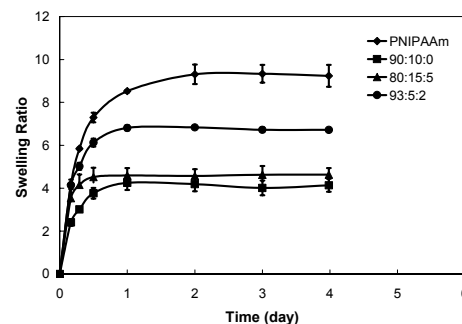


Figure 2 Hydrogel swelling profiles in PBS (pH 7.4) below the LCST at 25 °C

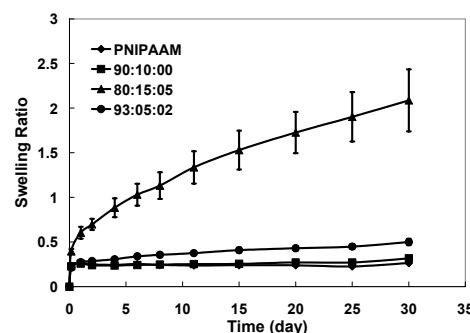


Figure 3 Hydrogel swelling profiles in PBS (pH 7.4) above the LCST at 37 °C

the NGF may bind with PNIPAAm or PLLA components, and this interaction tended to decrease the NGF release rate. However, the introduction of dextran component into the hydrogels could intensely reduce the interaction; therefore, NGF was released more rapidly at higher level from the dextran-incorporated hydrogels.

## Conclusions

A series of novel thermo-responsive and biodegradable hydrogels have been successfully synthesized by rational design techniques using PLLA as a hydrolytically biodegradable and hydrophobic unit, dextran as an enzymatically biodegradable and hydrophilic unit, and PNIPAAm as a thermo-responsive unit. The hydrogels show thermoresponsive properties and the LCST around 32 °C, typical to that of PNIPAAm. The hydrogels are also hydrolytically biodegradable with pore sizes increasing after about 4 months. The swelling behaviors of the hydrogels are different at temperature above (37 °C) and below (25 °C) the LCST and strongly depend on the hydrophilicity and hydrophobicity of the copolymers. The release profiles of NGF from the hydrogels are also different at temperatures above (37 °C) and below (25 °C) the LCST and depend on the swelling of the hydrogels and the interaction between the NGF and the hydrogels. In conclusion, the hydrogels have great potentials in controlled and sustained release of the NGF through changing their copolymer compositions, and thermo-responsive and biodegradable properties.

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Table 2 Cumulative NGF released in PBS (pH = 7.4) normalized by dry sample weight (ng NGF/mg dry gel)\*

Hydrogel	25 °C		37 °C	
	1 day	5 day	1 day	5 day
PNIPAAm	991 ± 70	1184 ± 83	116 ± 69	198 ± 141
90:10	758 ± 98	1054 ± 72	99 ± 42	151 ± 38
80:15:5	1031 ± 50	1492 ± 48	488 ± 166	690 ± 167
93:5:2	930 ± 64	1504 ± 110	125 ± 39	228 ± 56

\* Standard deviations are based on four experiments